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LETTER

Excessive lead burden among golden eagles in the Swiss Alps

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Abstract

Fragments from lead ammunition pose a poisoning risk for predators like golden eagles that scavenge on non-retrieved carcasses or offal left behind by hunters. Three golden eagles were found in the Swiss Alps with an acute lead poisoning. To investigate whether the few cases of lead-poisoned golden eagles are exceptional events or whether a substantial proportion of the Alpine golden eagle population is affected by lead at sublethal levels, we measured body burdens in golden eagles from Switzerland in comparison to eagle owls from the same area and to their respective prey. These two raptor species differ in their food as eagle owls feed on live-caught prey. Lead levels in soft tissues were significantly higher in golden eagles (median 1.14 $\mu g\,g^{-1}$ dry weight in liver, 0.99 $\mu g\,g^{-1}$ in kidney) than in eagle owls (0.14 and 0.23 $\mu g\,g^{-1}$). Bones of golden eagles contained 10 times more lead (median of 12.45 $\mu g\,g^{-1}$ dry weight) than owl bones (1.28 $\mu g\,g^{-1}$), which represent substantially higher levels than previously reported for golden eagles. Bones of prey of both golden eagles and eagle owls had low lead concentrations. In order to investigate whether the sublethal lead of golden eagles originates from ammunition or from generic environmental contamination, we examined lead isotope ratios. Lead isotope signatures of golden eagle bones were very similar to those of ammunition, but differed from the signatures of bones of their prey, eagle owls and soil. Isotope signatures did not change with increasing bone lead concentration in golden eagles or any other group examined. These findings indicate that in the Alps, most golden eagles take up lead from spent ammunition in carcasses or their offal in sublethal quantities throughout their life and a few in lethal quantities leading to acute lead poisoning.

1. Introduction

Poisoning by lead is recognized as a major threat to scavenging raptors and lead intake is still the most common toxic hazard occurring in raptors worldwide (Wayland *et al* 1999, Harmata and Restani 2013, Haig *et al* 2014). Lead poisoning is a special challenge to large predatory birds because their reproduction and mortality are naturally low and, therefore, the loss of a few individuals can seriously affect the survival of a population or even a species (Carpenter *et al* 2003, Finkelstein *et al* 2010, Lambertucci *et al* 2011). The major source of lead in terrestrially foraging raptor species was identified to be the ingestion of ammunition fragments embedded in wounded prey animals or non-removed carcasses or their offal which can lead to acute lead poisoning (Carpenter *et al* 2003, Church *et al* 2006, Fisher *et al* 2006, Martin *et al* 2008, Stansley and Murphy, 2011).

Acute lead poisoning is also known from golden eagles (*Aquila chrysaetos*) in North America (Craig *et al* 1990, Kramer and Redig, 1997, Wayland *et al* 1999), the UK (Pain *et al* 1995), Sweden (Kendall *et al* 1996) and Spain (Cerradelo *et al* 1992). In the Alps a few cases of lead-poisoned golden eagles are reported from Austria, Switzerland and Germany

(Bezzel and Fünfstück 1995, Zechner *et al* 2005, Kenntner *et al* 2007), and we add another three cases in this study. In all cases, ingestion of spent lead ammunition was suspected or found to be the reason for acute poisoning.

The question however remains, whether these few cases of acutely lead-poisoned golden eagles in the Alps are exceptional events or whether they represent the 'tip of the iceberg' of a substantial proportion of the Alpine golden eagle population affected by lead at sublethal levels. If so, a follow-up question is whether the sublethal lead of golden eagles originates from ammunition or from generic environmental contamination. In contrast to acute lead-poisoning, sublethal levels of lead in birds are much less well known (Haig *et al* 2014). Sublethal chronic lead assimilation may result in higher mortality or reduced reproduction (Pain *et al* 2009), potentially affecting a much higher proportion of the population than evidenced from individuals found with symptoms of acute lead poisoning.

In this study, we first explored the level of lead contamination in golden eagles of the Alps by performing a survey in birds found dead across eastern Switzerland. Second, we evaluated the sources of lead in these golden eagles. In the Alps, there are basically two possible sources of lead: a generic environmental contamination and consequent accumulation of the toxic heavy metal along the food chain, or the ingestion of lead from ammunition used for upland hunting. To distinguish between these two possible sources of the toxicant, we used three approaches.

First we compared the lead concentrations in tissues and bones of golden eagles with those of eagle owls. The rational basis of this side-by-side comparison between two predatory birds was that golden eagles, as frequent scavengers, are more exposed to lead shot and fragments contained in non-retrieved carcasses or offal than eagle owls which feed almost exclusively on live birds and small mammals killed with their powerful talons.

Second, we analysed the concentration of lead in bones of prey animals captured by golden eagle and eagle owl to see whether a distinct lead concentration in the two predators can be explained by different lead concentrations in their prey.

Third, we compared lead isotope ratios between soil, prey animals, golden eagles, eagle owls and ammunition. Isotope ratios have been shown to differ between ammunition and other sources and thus have been used to distinguish between different sources of lead (Finkelstein *et al* 2010, Walker *et al* 2013).

Our findings support the view that ammunition used for hunting upland game is not only a cause of mortality among scavenging birds of prey populations in Switzerland, but has lead to a substantial sublethal contamination with yet unknown consequences.

2. Material and methods

2.1. Sample collection

In this study we used all golden eagles and eagle owls we could get hold of, mostly through the Fish and Game Department of the Canton of Grisons. The 36 golden eagles were found dead, injured or moribund between 2006 and 2013. Most eagles were located in the Canton of Grisons ($N = 27$) and additional individuals in the Cantons of Berne ($N = 3$), Lucerne ($N = 2$), Glarus ($N = 2$) and Sankt Gallen ($N = 2$). From 31 of those, liver, kidney or bone samples were obtained, from 5 moribund only blood samples (supplementary data table S1, available at stacks.iop.org/ERL/10/034003/mmedia). The 19 eagle owls were from the Canton of Grisons except two eagle owls found in the Cantons of Zurich and Glarus. Most golden eagles were casualties of fatal intraspecific fights, the majority of owls died of electrocution on power lines or traffic. The age of the birds was determined from the stage of wing feather moult. Also, the animals were x-rayed to exclude that they were shot or had ingested bullet fragments. Blood was taken from six living golden eagles, one of which died. Liver, kidney and bones were collected from dead animals and all samples were stored at −20 °C.

We sampled bones of animals preyed by golden eagles ($N = 10$; mostly marmots *Marmota marmota*) and eagle owls ($N = 16$; birds, rodents, snow hares) which we found in or below their nests, hence they were not hunted (supplementary data table S2). We also sampled bones of 20 Alpine ibexes (*Capra ibex*) found dead in the wild, hence not hunted (supplementary data table S2), because golden eagles frequently feed on their carcasses, but their bones are rarely found in the nest, and because ibexes are also hunted.

Soil samples ($N = 20$) were taken from archived samples of the Cantonal (Department for Nature and Environment of the Canton of Grisons) and the Swiss National Soil Monitoring Network (NABO) (Meuli *et al* 2014), distributed over the whole area of the Canton of Grisons (supplementary data table S3). Three samples were from sediments of a lake contaminated by a historic, abandoned ore mine.

In addition we analysed the composition of lead isotopes in 16 shot pellets and bullets, two of them were retrieved from a dead golden eagle and a bearded vulture, respectively, and 14 were from ammunition commonly used in the study area for hunting upland game (supplementary data table S4).

2.2. Sample digestion and extraction

Blood samples were thawed and diluted with deionized water in a ratio of 1:10 for direct analysis. After thawing, the liver and kidney tissues were dried at 100 °C to constant weight, and on average 150 mg was analysed in duplicates. From bones (humerus, femur, sternum) adherent tissue was removed with a stainless

Table 1. Lead concentrations in lead-poisoned golden eagles and corresponding normal reference values from the literature (N.D., not determined).

	Liver (μg g^{-1} dry weight)		Kidney (μg g^{-1} dry weight)		Blood (μg dL^{-1})	
Bird	Measured	References[a]	Measured	References[a]	Measured	References[b]
GR7	77.35	<6 (>30 when poisoned)	30.88	<6 (>20 when poisoned)	N.D.	<20 (>50 when poisoned)
LU2	N.D.		N.D.		56.29	
SG1	N.D.		N.D.		108.0	

[a] Liver and kidney lead levels in golden eagles <6 μg g^{-1} dry weight indicate exposure to background lead concentrations, levels >6 μg g^{-1} are considered elevated, levels >30 μg g^{-1} in the liver and >20 μg g^{-1}in the kidney occur during lethal poisonings (Wayland *et al* 1999, Clark and Scheuhammer 2003).

[b] Blood concentrations <20 μg dL^{-1} are considered normal, levels >50 μg dL^{-1} occur during poisoning (Franson *et al* 1983, Garcia-Fernandez *et al* 1997, Pattee *et al* 2006, Stansley and Murphy 2011, Harmata and Restani 2013).

steel scalpel, the bones dried in an oven for 4 h to constant weight, and on average 250 mg was analysed in duplicates. A previous study (Ethier *et al* 2007) demonstrated that these and other bones accumulate lead to the same levels.

The tissue, bone, soil and ammunition samples were digested in closed Teflon® vessels using 4 mL of 65% (v/v) nitric acid (Suprapur, Merck) and 0.5 mL of 30% (v/v) hydrogen peroxide (Suprapur, Merck). Digestion was carried out in a Microwave System (UltraCLAVE, Milestone) with the heating program operating in three steps: (1) $t = 0$–10 min: temperature (T) increase to 220 °C, (2) $t = 10$–14 min: T increase from 220 to 250 °C, (3) $t = 14$–24 min: T at 250 °C. Pressure and energy were 160 bars and 1000 W, respectively, throughout the run. These treatments at high temperature were followed by cooling for 75 min. Due to incomplete digestion of soil, these samples were centrifuged (10 min at 4000 rpm) and decanted from silicate residues. All samples were transferred to plastic flasks and filled up to 50 mL with water followed by a dilution at a ratio of 1:5. One mL of the final solution was used for analyses.

2.3. Mass spectrometry

Lead concentrations were determined using an inductively coupled plasma mass spectrometer (ICP-MS) from Varian (Darmstadt, Germany). Calibration curves were prepared in aqueous solutions using ICP Multi Element Standard Solution XXI CertiPur (Merck). For quantifications, the average of lead isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb was used. Two different positive control samples served to verify the accuracy of the measurements: Human Hair Certified Reference Material No. 13 (National Institute for Environmental Studies, Japan) with a certified lead concentration of 4.6 ± 0.6 μg g^{-1}, and Seronorm™ Trace Elements Whole Blood at lead concentrations of 14.8 ± 1.0 and 336 ± 36 μg L^{-1}. Negative control samples were prepared in the same manner as each sample but without biological material. The limit of quantification for lead was 0.1 μg L^{-1}. Lead concentrations are expressed on a dry weight basis except for blood.

2.4. Data analysis

For the comparison of bone lead concentrations of golden eagles, eagle owls and their prey, we ln-transformed the values and used a Bayesian hierarchical version of the one-way ANOVA in order to cope with unequal variances and multiple comparisons. Posterior distributions of the group means were obtained by Monte-Carlo simulations using the package sim from the R-package arm (R Core Team 2014, Gelman and Hill 2007). Pairwise comparison between groups was done by calculating the posterior probability of the hypothesis that the difference between means is larger than zero.

Two isotope ratios were analysed statistically, because the third ratio is the product of the first two, thus does not contain any further information. To describe differences in the mean lead-ratios between the seven groups, we used a multivariate analysis of variance (MANOVA) with a bi-variate outcome variable. Because the variance in the measurements differed strongly between the different groups, we estimated different residual variances for each group. Therefore, we used a Bayesian MANOVA with heterogeneity of variances in OpenBUGS (Spiegelhalter *et al* 2007) with weakly informative priors (according to Gelman *et al* 2014). The two-dimensional posterior distribution of the mean lead isotope ratios for each group was described by 10 000 sampled pairs of values. From these values, we extracted the 95% confidence ellipses as two-dimensional credible intervals.

3. Results

Three golden eagles were found with signs of acute lead poisoning. They displayed high lead concentrations in the liver (77.4 μg g^{-1}), kidney (30.9 μg g^{-1}) or blood (56.3 and 108.0 μg g^{-1}) (table 1).

The comparison between systematically collected dead birds of the two raptor species (excluding those with acute intoxication signs reported in table 1) demonstrated that golden eagles displayed significantly higher lead concentrations than eagle owls in liver (figure 1(A)), kidney (figure 1(B)) and bone tissue (figure 1(C)). The lead content in the liver of

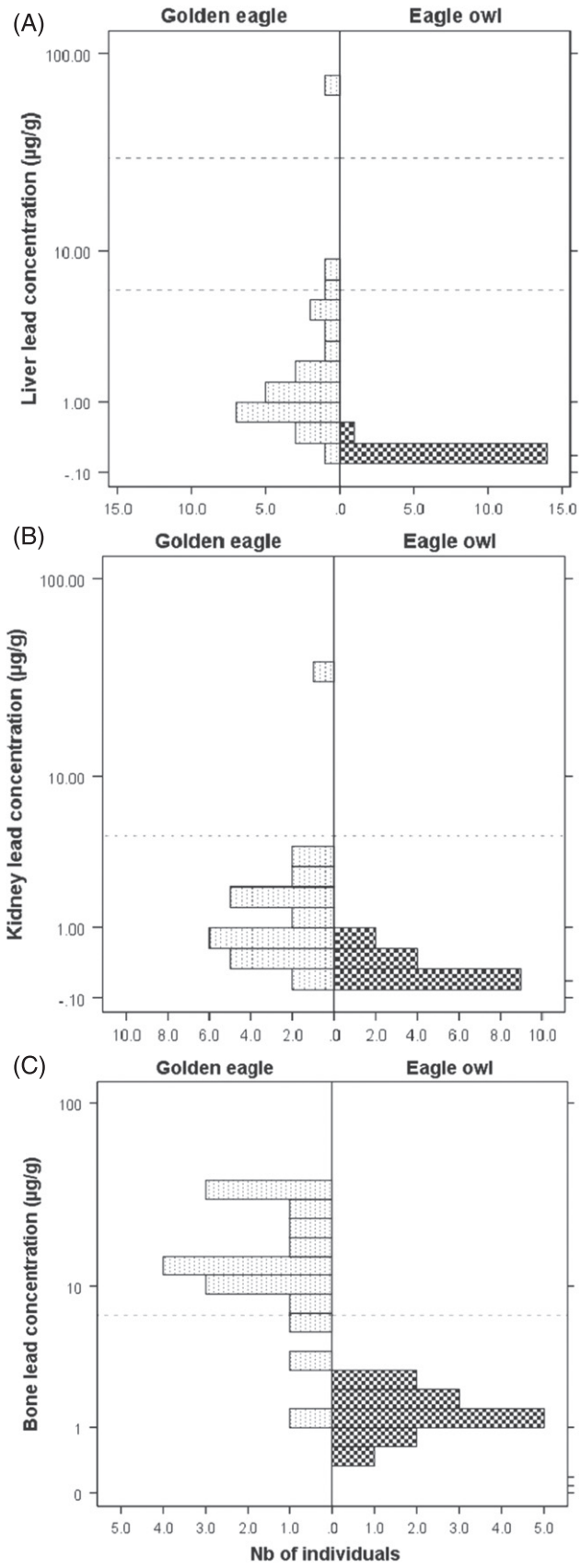

Figure 1. Frequency distribution of lead concentrations in the liver (A), kidney (B) and bones (C) of golden eagles and eagle owls. Note the logarithmic *y*-axis. Dotted lines indicate literature thresholds of elevated levels (6 μg g^{-1} for liver and kidney, 6.75 μg g^{-1} for bones), see table 1 and text. Lead concentration in liver, kidney and bones all differ significantly between the two species (non-parametric Mann–Whitney U-test, $p < 0.001$). In addition the high value for liver and kidney from table 1 is also shown.

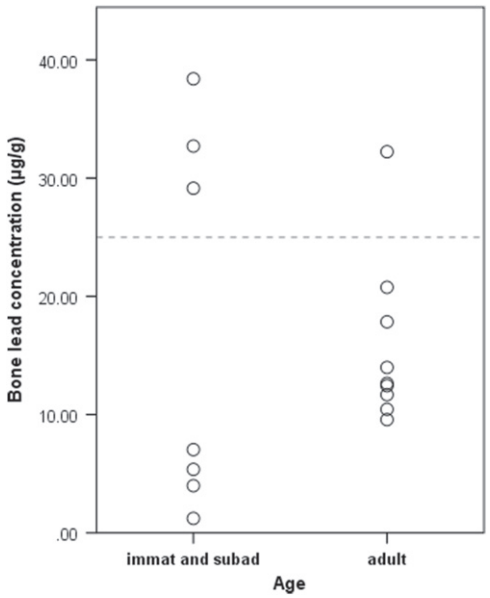

Figure 2. Bone lead concentrations of immature and subadult (<5 years old) versus adult (⩾5 years old) golden eagles. If the values >25 $\mu g\ g^{-1}$ are omitted, adult birds have significantly higher bone lead concentrations (9.58–32.23 $\mu g\ g^{-1}$, median 12.64 $\mu g\ g^{-1}$, $N = 9$) than the immature and subadult animals (1.22–7.04 $\mu g\ g^{-1}$, median 4.68 $\mu g\ g^{-1}$, $N = 4$, non-parametric Mann–Whitney U-test $p = 0.007$).

golden eagles ranged from 0.2 to 8.41 $\mu g\ g^{-1}$ with a median value of 1.14 $\mu g\ g^{-1}$ ($N = 25$). The respective concentrations in eagle owls ranged from 0.05 to 0.41 $\mu g\ g^{-1}$ with a median of only 0.14 $\mu g\ g^{-1}$ ($N = 15$). The lead content in the kidney of golden eagles ranged from 0.18 to 3.32 $\mu g\ g^{-1}$ with a median value of 0.99 $\mu g\ g^{-1}$ ($N = 24$). The respective concentrations in eagle owls ranged from 0.10 to 0.99 $\mu g\ g^{-1}$ with a median of only 0.23 $\mu g\ g^{-1}$ ($N = 15$). The largest difference between the two predatory bird species was detected upon analysis of their bones. In golden eagles, the lead content in this mineralized tissue ranged from 1.22 to 38.40 $\mu g\ g^{-1}$ (median concentration 12.45 $\mu g\ g^{-1}$, $N = 17$), whereas in eagle owls the lead content of the bones ranged from 0.46 to 2.29 $\mu g\ g^{-1}$ (median concentration 1.28 $\mu g\ g^{-1}$, $N = 13$). Only the lowest golden eagle value was within the range of eagle owl values.

Lead incorporated in the bones is thought to integrate all episodes of exposure within the life of an individual. Therefore, due to the continued accumulation of this heavy metal in mineralized tissues, the lead content of bones is expected to steadily increase with age (Rodriguez-Ramos Fernandez *et al* 2011). However, among the golden eagles we observed an inverse trend of lead concentrations in the bones as the animals progress from juvenile and sub-adult periods of life (<5 years old) to the adult stage (⩾5 years old; figure 2). In particular, the younger birds could be clearly separated in a group with low lead concentrations in the bones and another group with increased lead burden (>25 $\mu g\ g^{-1}$). This pattern of lead accumulation may indicate that young golden eagles with lead contents in bone exceeding 25 $\mu g\ g^{-1}$ may suffer from increased mortality. This view is confirmed by the observation that the expected age-dependent increase of lead concentrations in the bones is re-established when the birds with values >25 $\mu g\ g^{-1}$ are omitted from the calculations (figure 2).

The concentration of lead in bones of prey animals found in golden eagle and eagle owl nests (mean ± SD 2.56 $\mu g\ g^{-1} \pm 1.35$ for golden eagle prey and 2.95 $\mu g\ g^{-1} \pm 0.63$ for eagle owl prey) was indistinguishable from that of eagle owl bones, but significantly lower than the values of golden eagle bones (figure 3). Lead concentration in bones of Alpine ibexes was significantly lower than in prey animals or eagle owls (figure 3). There was no significant correlation between lead concentration in bones and the age of Alpine ibexes ($p = 0.86$; linear regression).

The isotope ratios of $^{207}Pb/^{208}Pb$ and $^{206}Pb/^{208}Pb$ found in the bones of golden eagles were statistically indistinguishable from those determined for ammunition (figure 4). The isotope ratios in bones of prey animals of the golden eagle, ibexes, eagle owls and in soil samples were significantly different from the lead isotope ratios of both golden eagles and ammunition (figure 4). Prey animals of eagle owls were intermediate and statistically indistinguishable in their Pb isotope ratios from ammunition, golden eagles and eagle owl bones. In detail, the isotope ratios in bones of Alpine ibexes and of golden eagle prey animals are similar among each other. Eagle owl bones showed a large variation in both Pb isotope ratios and were statistically different from bones of golden eagle prey, ibexes and golden eagles and from ammunition. Soil samples showed a very small variation in Pb isotope ratios and differed significantly from ammunition, and bones of golden eagles, golden eagle prey, eagle owl prey (figure 4).

There was no correlation between the lead isotope ratios and the lead concentration in bones of golden eagles ($p = 0.27$ for $^{207}Pb/^{208}Pb$ and $p = 0.34$ for $^{206}Pb/^{208}Pb$, linear regression) or in bones of eagle owls, their prey, Alpine ibex (taken together or separately) or soil samples ($p > 0.12$ in all analyses, linear regression). In particular, the three soil samples from sediments of a lake contaminated by the abandoned ore mine showed very high lead concentrations (118–503 $\mu g\ g^{-1}$), but their isotope ratios did not differ from the other soil samples (supplementary data table S3).

4. Discussion

In this study, we found three cases of golden eagles in the Alps with acute signs of lead poisoning, confirmed by excessive concentrations of lead in blood, liver and kidney (table 1). Once absorbed in the small intestine, lead binds to red blood cells before being redistributed

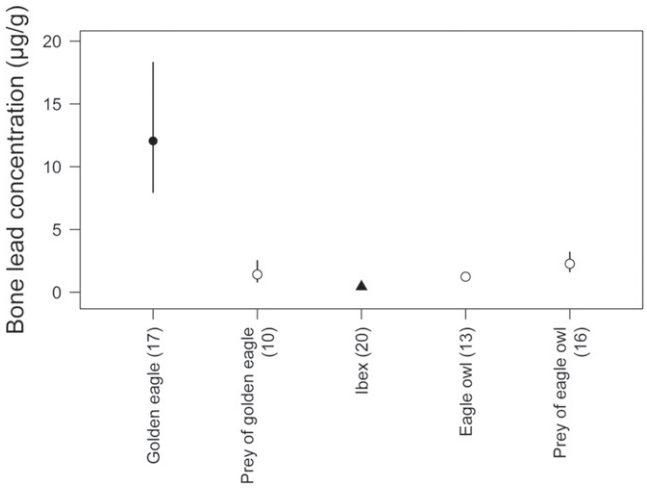

Figure 3. Mean bone lead concentration of golden eagles and eagle owls compared with their prey. Lines are 95% confidence intervals (if not shown they are smaller than the symbol). Means not sharing the same symbol are significantly different from each other at the $p < 0.001$ level. Numbers in parentheses are sample sizes.

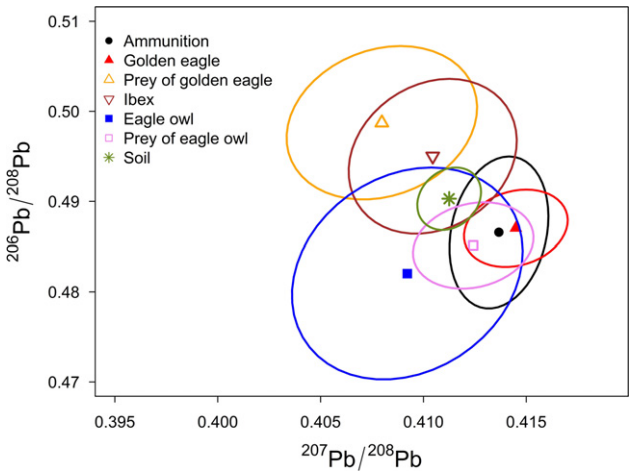

Figure 4. Mean ratio of $^{207}Pb/^{208}Pb$ and $^{206}Pb/^{208}Pb$ found in bones of golden eagles, eagle owls, their prey and in Alpine ibex, as well as in ammunition and soil. Ellipses are 95% confidence intervals. A sample is significantly different from another sample if the mean is not included within the 95% confidence ellipse of the other sample.

to highly perfused organs. First, there is a rapid exchange between the circulating blood and the parenchyma of liver, kidney and the brain (Nam *et al* 2012). By replacement of calcium ions, this heavy metal is then slowly transferred into the bones, which ultimately contain >90% of the overall body burden. Whereas lead in liver and kidney has a turnover rate of weeks to months, bones constitute a long-term depot (Ethier *et al* 2007, Rodriguez-Ramos Fernandez *et al* 2011). As a consequence, lead levels in blood are measured for the diagnosis of poisoning in living birds, whereas analyses of liver and kidney are used for the post-mortem confirmation (see table 1).

The concentrations of lead in the bones of raptors are usually not directly related to current episodes of exposure (Ethier *et al* 2007, Gangoso *et al* 2009), but can increase rapidly after lead ingestion (Sanderson and Bellrose 1986). Concentrations >10 $\mu g\,g^{-1}$ in bone tissue are considered to be elevated and concentrations >20 $\mu g\,g^{-1}$ are often observed after lethal poisoning (Mateo *et al* 2003, Rodriguez-Ramos Fernandez *et al* 2011). Interestingly, the lead concentrations in the bones of golden eagles found in our study (median of 12.45 $\mu g\,g^{-1}$ dry weight) were substantially higher than those previously reported in the literature for the same species. For example, Mateo *et al* (2003) detected bone concentrations between 0.49 and 4.97 $\mu g\,g^{-1}$ dry weight in golden eagles found in Spain. Wayland *et al* (1999) divided the birds into those with background hepatic lead levels (<6 $\mu g\,g^{-1}$) and those with elevated hepatic levels (>6 $\mu g\,g^{-1}$). Mean bone lead concentrations in this Canadian study were 4.1 $\mu g\,g^{-1}$ for the former group and 9.7 $\mu g\,g^{-1}$ for the latter. Clark and Scheuhammer (2003) found in Canada that 5 out of 9 birds had bone lead concentrations >6.75 $\mu g\,g^{-1}$, whereas we found in our study a

higher proportion (14 out of 17 specimens) with concentrations in the bone >6.75 $\mu g\,g^{-1}$.

Other scavenging raptors are also likely to take up lead (see Rodriguez-Ramos Fernandez *et al* 2011). Among four examined bearded vultures from the Alps, only one had a low lead concentration in bones (6.50 $\mu g\,g^{-1}$), while three had very high values (38.90–100.04 $\mu g\,g^{-1}$; Bassi *et al* (2013), Bassi *et al* in press, own unpublished data). Hence, the bone lead concentrations found in systematically collected dead golden eagles in the eastern Swiss Alps was exceptionally high compared to those found in other golden eagle populations. In liver and kidney, lead concentrations of golden eagles without signs of lead intoxication did not exceed literature thresholds of elevated levels, but were significantly higher than in eagle owls. Hence, although no signs of acute poisoning were apparent, even kidney and liver levels were elevated compared to another predator bird.

Lead concentrations in bones of golden eagle prey animals were >10-fold lower than in golden eagle bones and ibex bones contained even >20-fold less lead (the median of 0.47 $\mu g\,g^{-1}$ found in this study compares well with the median of 0.59 $\mu g\,g^{-1}$ found by Tataruch and Onderscheka (1995) in the same area). In contrast, bones of eagle owl prey contained even slightly more lead than eagle owl bones. Therefore, the large difference in bone lead concentration between the two predators cannot be explained by differences in lead concentration of their prey. Part of the difference between golden eagles and eagle owls in bone lead concentration may be due to the fact that gastric pH values of owls are not quite as low as in raptors (Fisher *et al* 2006, Rodriguez-Ramos Fernandez *et al* 2011) and that owls regurgitate bones, while raptors partly digest and partly regurgitate bones. Hence, the absorption of ingested lead may be more complete in raptors than in owls.

The examination of lead isotope ratios revealed that lead of golden eagles was most similar to that of ammunition used in the study area and differed from the isotope signature of golden eagle prey and soil samples (even those with high lead concentrations from ore mines or natural occurrence). Surprisingly, there was no change in isotope signature with increasing lead concentration in bones of golden eagles (or any other sample). This indicates that the source of lead, i.e. from ammunition, remains the same irrespective of the amount of lead up-take.

Taken together, our findings indicate that lead in golden eagles originates from ammunition and is not the consequence of a bioaccumulation of generically available lead in the environment (e.g. from old ore mine or aerial deposition of industrial lead). The finding that lead is accumulated in very high amounts in bones of golden eagles and is present in higher amounts than in another predator species, even in liver and kidney, suggests a frequent uptake of sublethal amounts of lead. The lead concentration in segments of wing feathers showed an irregular pattern, indicating an episodic rather than continuous lead uptake (own unpublished data).

There are two main sources of ammunition lead for golden eagles in the Alps which cannot be distinguished by this study. First, golden eagles could feed on animals shot with lead pellets which were not retrieved or whose carcasses were left in the environment (e.g. fox carcasses after skinning). Second, golden eagles could take up lead fragments from animals, or their offal, shot with lead bullets, still the main ammunition of hunters in the study area.

When striking their targets, conventional lead-based rifle bullets partly disintegrate into metallic fragments, causing a widespread contamination of animal tissues (Church *et al* 2006, Hunt *et al* 2006, Bassi and Ferloni 2012, Trinogga *et al* 2013, Haig *et al* 2014). Since golden eagles frequently prey upon weak, moribund or dead animals, they undergo an increased exposure to lead shot or bullet fragments enclosed in the flesh of hunter-crippled game and prey carcasses. It is also general practice to eviscerate hunted wildlife in the field, thereby leaving behind lead-contaminated offal (digestive tract, heart, lungs) that may be readily ingested by terrestrial raptors, if not buried properly. During the hunting season, about 10 000 shot red deer, roe deer, chamois and ibex provide about 100 tons of offal of which part may not be properly buried and therefore available to the 120 breeding pairs of golden eagles in the Grisons. The oral uptake of lead shot and bullet fragments from these sources may cause severe poisonings (Garcia-Fernandez *et al* 1997, Pattee *et al* 2006, Stansley and Murphy 2011). Other possible routes of lead uptake seem much less likely. Nonlethal lead shot at golden eagles and incorporated into the body has been found only in one case. Lead emissions during military exercises is not available to the birds together with their food and are expected to contribute minimally to the lead burden of golden eagles.

We conclude that, in the Alps, most golden eagles take up lead from spent ammunition in carcasses or their offal in sublethal quantities throughout their life (see the high bone levels of almost all eagles examined in this study) and a few in lethal quantities leading to acute lead poisoning (see table 1 and Bezzel and Fünfstück 1995, Zechner *et al* 2005, Kenntner *et al* 2007).

Besides causing direct mortality through the ingestion of high amounts of lead, the intake of sublethal amounts may affect avian populations by altering cognition and behaviour, diminishing reproductive success and causing diseases as well as starvation or traumatic events, or by subjecting the exposed birds to undue predation (Gangoso *et al* 2009, Rodriguez-Ramos Fernandez *et al* 2011). The successive accumulation of larger sublethal quantities early in life may indeed have resulted in a higher mortality (see figure 2). However, very little is known about the effects of sublethal lead burdens in any wild bird

population (Haig *et al* 2014). Although the golden eagle population in the study area has increased in the last 60 years (e.g. +10% since the census in 1990–1992, Haller (1996)) and prosper at high density, the introduction of lead-free ammunition for upland hunting in the investigated Alpine regions would greatly reduce the overall lead burden and contribute to the health of scavenging raptors in general. This would be particularly important for bearded vultures which are being re-introduced from captive breeding programs and still represent a very small and vulnerable, although increasing, population in the Alps (Schaub *et al* 2009). Their very high bone lead concentrations (see above) need further attention.

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